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Virulence Evaluation of *Aeromonas* spp. KS-1 Isolated from Kitchen Sponge using *Omphisa fuscidentalis* Larvae

Noor Andryan Ilsan[1*], Maulin Inggraini[1], Siti Nurfajriah[1], Melda Yunita[2], Jepri Agung Priyanto[3], Viqih Ramanda[4]

[1]*Department of Medical Laboratory Technology, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga, Bekasi 17113, Indonesia*
[2]*Department of Microbiology and Parasitology, Faculty of Medicine, Universitas Pattimura, Ambon, Maluku, Indonesia*
[3]*Division of Microbiology, Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, Bogor 16680, Indonesia*
[4]*Vocational Student, Department of Medical Laboratory Technology, Sekolah Tinggi Ilmu Kesehatan Mitra Keluarga, Bekasi 17113, Indonesia*

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ABSTRACT

***Aeromonas* spp. causes the human diseases including diarrhea, gastroenteritis, and bacteremia. *Aeromonas* spp. can be found in kitchen sponge, one of the reservoirs for food-borne bacterial pathogens. Virulence study of *Aeromonas* spp. *in vivo* in animal model is important since the animal model can mimic manifestasions in human infections. *Omphisa fuscidentalis* was chosen for alternative virulence model, since they are in the same taxonomical order with the well-known infections model, *Galleria mellonella*. Bacterial isolation and selection of kitchen sponge used Brain Heart Infusion agar and Endo Agar, respectively. Bacterial virulence of KS-1 was injected into *Omphisa fuscidentalis* larvae. Survival percentage and melanization score of infected larvae were evaluated. Hemolymph of larvae with melanization score of 1 and 4 were stained with Giemsa method to observe the hemocyte changes. Bacterial identification of isolate KS-1 based on 16S rRNA sequence resulted in 96.9% identity to *Aeromonas* spp. strain VS7. Isolate KS-1 injection to *O. fuscidentalis* revealed higher bacterial dosage resulting more severe symptoms to the larvae according to survival percentage and melanization score. However, statistical analysis showed evaluation of melanization score could distinguish larvae with 10^6 and 10^7 CFU/larva dosage injection, while evaluation of survival percentage could not. Hemocyte of larvae with melanization score 1 had larger and more cytoplasmic vacuolization than the score 4 (healthy larvae). *Omphisa fuscidentalis* is an alternative of insect model for bacterial infections with survival percentage and melanization score as the evaluation. Cytoplasmic vacuolization of hemocyte can be used as larvae's health indicator in a cellular level.**

1. Introduction

Genus *Aeromonas* is a Gram-negative bacterium that belongs to Aeromonadaceae family. These bacteria have many been found in freshwater, food product, fresh meat and vegetables. *Aeromonas* spp. can cause disease in humans, animals, particularly fish and other fresh water animals. In human, *Aeromonas* spp. cause bacteremia, diarrhea, gastroenteritis, and wound infections. Recent clinical study using 1852 strains as samples, showed four dominated species of *Aeromonas* spp., namely *Aeromonas* caviae, *A. dhakensis*, *A. veronii*, *A. hydrophila* with 37.3%, 23.5%, 21.5%, 13.1%, respectively (Fernandez-Bravo and Figueras 2020). Several factors responsible to *Aeromonas* virulence are toxin gene (*exoA*, *alt*, *act*), structural genes (*flaA*, *maf-5*, *flp*), and secretive protein system (*T3SS*, *T6SS*) (Beaz-Hidalgo and Figueras 2013). The *A. caviae* is the highest prevalence clinical species among genus *Aeromonas*. *Aeromonas* spp. can be found in many specimens including urine, faeces, bilis, wound, abscess blood, ascitis, and respiratory tract (Fernandez-Bravo and Figueras 2020). Moreover, *Aeromonas* spp. is also widely distributed in many non-human habitats such as food and animals. A study revealed that *A. caviae* and *A. hydrophila* have

* Corresponding Author
E-mail Address: noor.andryan@stikesmitrakeluarga.ac.id

been known to attach, invade, and release cytotoxic in human larynx Hep-2 and human Caucasian colon carcinoma Caco-2 cells (Krzyminska *et al.* 2012; Merino *et al.* 2014; dos Santos *et al.* 2015). A study in 2013 of Japan isolates reported that *A. caviae* was the most common isolates presented in patients with a solid tumor, compared with non-A. caviae isolates. The percentage of overall 30-day mortality was 19% (Kimura *et al.* 2012).

Kitchen sponge is one of source of abundant bacteria in household tools (Osaili *et al.* 2016). Kitchen sponge can disseminate the bacteria as the residue of cleaning the food utensils that can be the factor of bacterial cross contamination of foodborne transmitted infections (Wolde and Bacha 2016). Inappropriate handling, improper storage, and long duration usage, lead to survival and growth of microbial in the kitchen sponge, including *E. coli, Staphylococcus* spp., *Salmonella* spp., *Klebsiella* spp., *Enterobacter cloacae*, and *Cronobacter sakazakii* (Rossvoll *et al.* 2015; Cardinale *et al.* 2017; Marotta *et al.* 2018; Inggraini *et al.* 2021; Ilsan *et al.* 2022). Although *Aeromonas* spp. is categorized as a human pathogen, but less studies of bacterial virulence evaluation of *Aeromonas* spp. Virulence evaluation of *A. caviae* using animal model was studied in mice (*Mus musculus*) and nematode *Caenorhabditis elegans* (Chen *et al.* 2014, 2018). Insect larvae of *Galleria mellonella* is an alternative model for evaluating bacterial virulence, especially human pathogens due to optimal growth in 37°C. Insect larvae has an analogue to human phagocytic cells i.e. hemocyte (Tsai *et al.* 2016). However, *G. mellonella* in Indonesia is not sold commercially and difficult to find in nature. In this study, we used *Omphisa fuscidentalis* larvae as an insect model for evaluation of *Aeromonas* spp. from kitchen sponge. *O. fuscidentalis* has the same taxonomic order with *G. mellonella*. The bacterial evaluation assessed included survival rate, melanization level, and macroscopic observation of hemocyte. In our knowledge, this is the first study of bacterial virulence of human pathogen evaluation using *O. fuscidentalis*.

2. Materials and Methods

2.1. Bacterial Isolation of Kitchen Sponge

Kitchen sponge has been used in 2 weeks for washing the family dishes, was swabbed using a sterile cotton swab. Bacterial colonies were isolated using spread plate method in several dilution. Brain Heart Infusion agar (HiMedia) was used for growing the bacteria (Marotta *et al.* 2018). Grown colonies were cultured further onto Endo agar (HiMedia), as a differential and selective medium. Bacterial culture was incubated in 37°C for overnight (Memmert IN55, Germany). One predominant colony grown on Endo agar was selected for molecular identification and virulence evaluation. All of the procedures were performed in JSCB-900SB Biosafety Cabinet (JSR, Gongju, South Korea).

2.2. Molecular Identification of KS-1 and Phylogenetic Tree Construction

Isolate KS-1 was a colony boiled at 98°C for 15 minutes in thermal cycler. The colony boiled of KS-1 was then subjected as a template for Polymerase Chain Reaction (PCR) amplification of partial sequence of 16S rRNA using primer of 1387r (5' GGGCGGWGTGTACAAGGC 3') and primer of 63f (5'CAGGCCTAACACATGCAAGTC 3') (Marchesi *et al.* 1998). The PCR amplicon product was 1,300 bp. As 50 µtotal reaction of PCR mixture was prepared using the following components: 25 µl GoTaq Green Master Mix (Promega, Madison, WI, USA), 5 µl primers 1387r (10 pmol), 63f (10 pmol), 4 µl KS-1 genomic DNA that had been boiled in a colony, and 11 µl nuclease-free water. Thirty cycles of pre-denaturation at 94°C for five minutes, denaturation at 94°C for thirty seconds, annealing at 55°C for four seconds, and elongation at 72°C for ten minutes were used to condition the PCR. DNA product separation was accomplished using 1.5% agarose gel. The separation of DNA band was observed using a UV transilluminator. The PCR product was then sequenced at 1st Base Genetika Science in Indonesia using the Sanger method. MEGA X software was used to trim and assemble the forward and reverse primer sequences. The assembled sequence was aligned to the closest reference using the BLASTN algorithm on the National Center for Biotechnology Information (NCBI) website. A phylogenetic tree was built using Mega X version 11 with the Maximum Likelihood statistical technique. Using the Find Best Model menu, the best analytic model for all partial 16S rRNA sequences was determined, and Tamura-Nei (TN93) was found to be an appropriate model.

2.3. Preparation of *Aeromonas* spp. Culture for *O. fuscidentalis* Injection

Aeromonas spp. was cultured onto BHI agar. Sub-cultured grown colonies were placed in 2 ml sterile BHI broth, it was then incubated in 100 RPM shaker

for overnight. One ml broth culture was centrifuged in 8,000 RPM for 5 minutes at room temperature. The pellet was resuspended in 500 µl of sterile phosphate buffer saline (PBS), and the supernatant was disposed of. Thermo Fisher Scientific, Waltham, Massachusetts, USA, used a Genesys 10S UV-Vis spectrophotometer to determine the absorbance of the bacterial culture in OD_{600}. PBS was used to adjust the bacterial suspension to OD=1, or around 10^9 CFU/ml of bacterial density. Bacterial dosages used were 10^7, 10^6, 10^5, 10^4 CFU/larva.

2.4. *Aeromonas* spp. Injection in *O. fuscidentalis* Larvae as an Infection Model

Acinetobacter baumannii-infected *Galleria mellonella* larvae were used in the bacterial injection approach, which referred to Ilsan *et al.* (Ilsan *et al.* 2021, 2023). The larvae of *O. fuscidentalis* were reared in homegrown. In this investigation, late phase *O. fuscidentalis* larvae weighing 300–400 grams were used. For every group, ten larval duplicates were injected, one of which was a PBS-injected control. The larvae were put on 70% alcohol tissues prior to injection. The last left proleg of the larvae was injected with 10 µl of bacterial solution. The JSCB-900SB Biosafety Cabinet (JSR, Gongju, South Korea) was used for all injection procedures. Larvae were injected and then incubated for 8 and 24 hours at 37°C. The melanization score and survival percentage were assessed using the following categories: score 0 (dead, complete melanization with full black color), score 2 (tail/line melanization, brown color), score 1 (spots melanization, blackish color), and score 4 (healthy, no melanization) (Tsai *et al.* 2016). By squeezing the larvae that had been cut with a sterile scalpel, the hemolymph of larvae with melanization scores of 1 and 4 was collected. After staining 10 µl of larvae hemolymph using the Giemsa procedure, the hemocyte cells were examined under a light microscope. GraphPad Prism 5 was used to create the Kaplan-Meier survival curve (GraphPad, San Diego, CA, USA). GraphPad Prism 5 was used to examine the Kaplan-Meier survival curve using log-rank statistical analysis. Statistical significance of melanization score was confirmed by One-Way ANOVA using GraphPad Prism 5.

3. Results

One bacterial isolate was randomly selected from various bacteria grown in Endo agar, namely KS-1. The molecular identification showed KS-1 was closest identity to *Aeromonas* caviae strain VS7 (96.9% identity) based on 1057 base pairs of assembled sequence comparison with NCBI accession number MG062856.1. Partial sequences of 16S rRNA of KS-1 was deposited to NCBI with accession number OP010008. KS-1 was evaluated further for bacterial virulence evaluation in *Omphisa fuscidentalis*. BLASTn result of clean contig of partial 16S rRNA KS-1 isolate showed several genera *Aeromonas* with closer identity, including *A. hydrophila* T50-2, *A. punctata* WAB 1957, *A. dhakensis* W2-2, *A. caviae* IR11, and *E. coli* ATCC 25922 as a Gram negative outspecies. All of these sequences were chosen for phylogenetic tree construction (Figure 1).

KS-1 culture injection in *O. fuscidentalis* showed that 10^7 CFU/larva dosage was the most lethal to the larvae with 30% survival in 5 hours, and 20% in 24 hours post-injected. In 10^6 CFU/larva, survival rate reached 90% in 5 hours and 30% in 24 hours. Meanwhile, 10^4 and 10^5 CFU/larva showed the same survival percentage with 100% in 5 hours and 50% in 24 hours. In PBS-injected larvae as a control negative, all larvae survived in 5 hours, while 80% survived in 24 hours (Figure 2). In 5 hours post-injected, all dead larvae were appeared as fully or partially black, as the initial melanization (Figure 3). However, some of living larvae has partial melanization. Therefore, using *G. mellonella* as a bacterial infections model, was referred for evaluating the melanization score. Melanization score evaluated in 5 hours, was more reliable incubation time, since the the dead in PBS-injected larvae was found in 24 hours. According to melanization score, the rank of healthy larvae is similar with those survival percentage evaluation. However, a higher resolution in melanization score was occured than the survival percentage, in terms of healthy status of the larvae. Statistical analysis indicated no significant difference in survival percentage between 10^5 and 10^6 CFU/larva, 10^6 and 10^7 CFU/larva. Meanwhile 10^5 and 10^7 CFU/larva were significant different with $p = 0.066$ (Figure 2). However, the evaluation showed a higher resolution in melanization score. Only 10^5 and 10^6 CFU/larva that had no significant difference. Meanwhile, 10^6 and 10^7 CFU/larva, 10^5 with 10^7 CFU/larva showed significant different with $p<0.001$ (Figure 4). Hemocyte of *O. fuscidentalis* was evaluated using Giemsa staining. Result showed that larvae with melanization score 1 (alive, weak, high melanization), had relatively a higher number

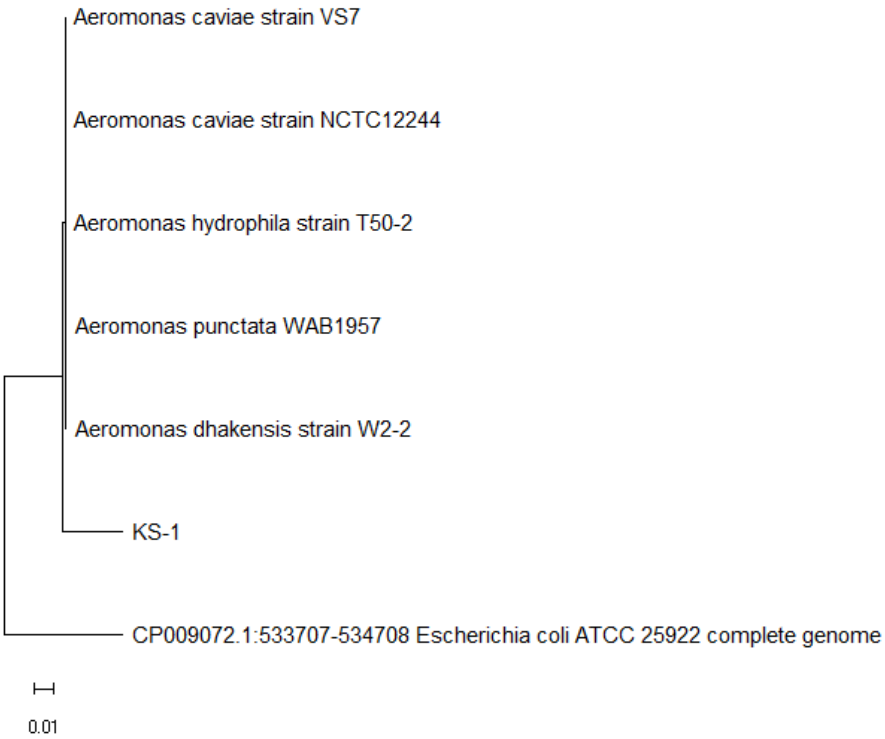

Figure 1. Phylogenetic tree of *Aeromonas* spp. KS-1 of partial gene of 16S rRNA sequence, with another closer identity genus *Aeromonas. E. coli* ATCC 25922 has been chosen as an outspecies for Gram negative bacteria

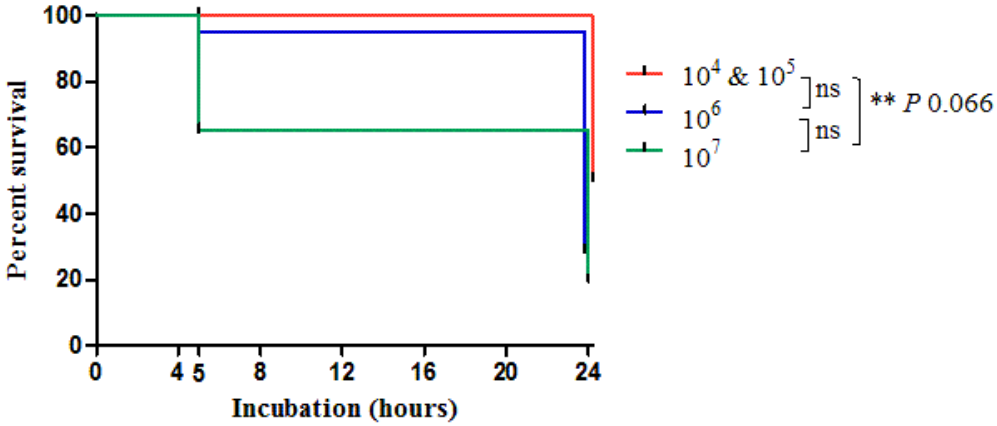

Figure 2. Kaplan-Meier survival curve of *O. fuscidentalis* was infected by *Aeromonas* spp. KS-1 in 5 and 24 hours incubation. *means p≤0.05, **means p≤0.01, ***means p≤0.001. ns means not significant

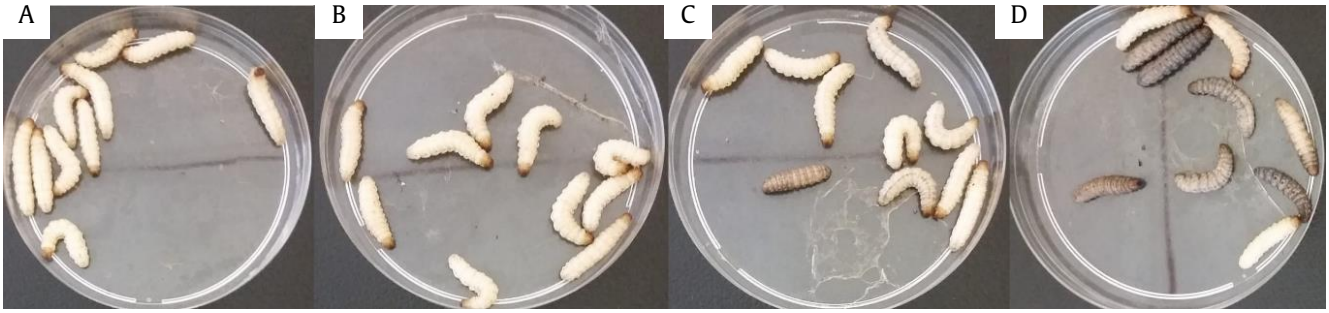

Figure 3. Melanization display of *O. fuscidentalis* larvae in several dosage of 5 hours after injection. Melanization was seen as blackness in skin and hemolymph. (A) 10^4 CFU/larvae, (B) 10^5 CFU/larvae, (C) 10^6 CFU/larvae, (D) 10^7 CFU/larvae

and larger vacuolization in hemocyte, than those PBS-injected larvae score 4 (alive, no melanization) (Figure 5). Vacuolization was localized in cytoplasma.

4. Discussion

Kitchen sponge is a household tool that can become the domestic source of infections for food-borne disease. The awareness of domestic home in food-borne disease dissemination is considered very low. Various items consumed after dishwashing can be the source of bacteria in kitchen sponges. A study in Italy showed a wide variety of species found in kitchen sponge, including aerobic mesophilic bacteria, *Enterobacteriaceae*, yeasts and molds, *Anaerobic bacteria, Micrococci, Staphylococci, Salmonella, Yersinia enterocolitica*, and *Lysteria monocytogenes*. Identification of *Enterobacteriaceae* group using Matrix-assisted laser desorption ionization-time flight mass spectrometry (MALDI-TOF MS) revealed there was opportunistic and pathogenic bacteria such as *Enterobacter cloacae*, *Citrobacter freundii*, and *Cronobacter sakazakii*. In addition, there were strains inlcuding *K. pneumoniae, K. oxytoca*, and *C. sakazakii* categorized as Extended-spectrum beta lactamase (ESBL) producing (Marotta *et al.* 2018).

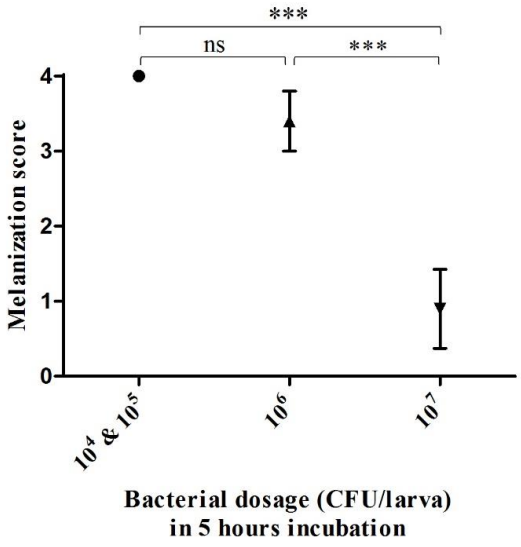

Figure 4. Melanization score of ten *O. fuscidentalis* for each bacterial dosage of *Aeromonas* spp. KS-1. *means p≤0.05, **means p≤0.01, ***means p≤0.001. ns means not significant

A study conducted in United Arab Emirates in 2020 (Osaili *et al.* 2016) using kitchen sponge from university dormitory as sample, identified *Enterobacteriaceae* including *E. cloacae, E. aerogenes, K. pneumoniae, K. oxytoca*, and *Serratia mercescens*. In addition of isolated mesophilic aerobic bacteria, coliform, yeasts and molds. Vitek 2 was used for biochemical identification and antimicrobial susceptibility test in this study. Interestingly, all *E. cloacae* isolates were resistant to several antibiotics including cefuroxime axetil, amoxicillin, cefalotin, and cefoxitin (Osaili *et al.* 2016). From those study, many harmful pathogenic bacteria has been found in kitchen sponge. However, more investigation of bacterial virulence from kitchen sponge is required, particularly on their pathogenicity in an animal model.

Bacterial virulence is an important aspect to evaluate the pathogenesis, namely disease compensation in the host. Animal is a reliable model for evaluating *in vivo* to mimic the disease development of human pathogenic bacteria. Several animals have been known as good models for evaluating bacterial infections, such as mice,

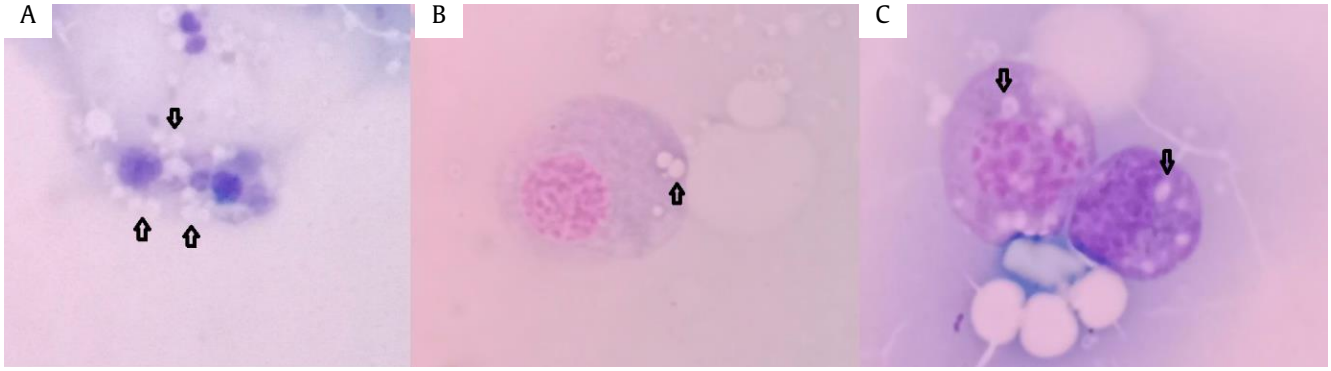

Figure 5. Giemsa staining of *O. fuscidentalis* larvae hemocyte from melanization score 1 (A) and score 4 (B and C). Hemocyte from melanization score 1 showed more and bigger vacuolization in cytoplasma (A) than score 4 (B and C). Darker color in the center is nucleus. Vacuolization appears white bubble in their cytoplasma (shows by arrow)

zebra fish, nematode *Caenorhabditis elegans*, and insect larvae *Galleria mellonella*. Mice is a popular model since it is mammalian model, has many similar organs to human. However, mice needs a highly maintenance action to obtain optimal condition, with expensive cost, long reproductive period, long disease progression time, requiring an ethical approval before being used. Zebra fish is an alternative model for bacterial infections. It should be noted that fish model needs special tools to maintain the growth and nowadays, ethical approval should be obtained for experiment using zebra fish. Nematode *C. elegans* has been known as an infection model. Nevertheless, this nematode can be maintained in 37°C because this model requires lower temperature for optimum growth. For bacterial infections, insect larvae is becoming a good alternative model, since it is cheap, no need of ethical approval, able to grow in 37°C, has short reproductive and disease progression time, also as many as larvae replication can be used to obtained a strong statistical conclusion (Cook and McArthur 2013; Tsai *et al.* 2016; Kavanagh and Sheehan 2018). *Galleria mellonella* is a well-known model in bacterial infections of lepidoptera order. Besides studies about bacterial infections, *G. mellonella* was used to evaluate the antimicrobial activity *in vivo* against bacterial pathogens. Maekewa et al (Maekawa *et al.* 2015) have been reported various extract of *Zingiber officinale* (fresh extract, dried extract, and glycolic extract) could decrease *Enterococcus faecalis* infections. The survival percentage became the only one evaluation for disease development. A study of using glycolic extract of *Punica granatum* (Pomegranate) revealed antimicrobial activity *in vivo* in *G. mellonella* larvae against periodontal pathogen Porphyromonas gingivalis (Gomes *et al.* 2016). A decrease survival rate was showed in low glycolic extract of *P. granatum*, including 12.5, 6.25, 3.1, and 2.5 mg/ml. While higher dose of injection (200, 100, and 25 mg/ml) showed toxic effect, leading to death. There are more than 1,000 publications about *G. mellonella* as an animal model in PubMed (Tsai *et al.* 2016). Since, *G. mellonella* is a beehive pest of *Apis* sp., *G. mellonella* larva is difficult to obtain either in environment or in the store/pet shop in Indonesia. Thus, *Omphisa fuscidentalis* has a similar taxonomy order with *G. mellonella*, and this larva has commercialized in several stores in Indonesia can be used as alternative.

In this study, *Aeromonas* spp. was found in 2 weeks-used domestic household kitchen sponge. *A. caviae* is one of important *Aeromonas* genus and ubiquitously found in diverse source, especially human specimens. Using ERIC-PCR genotyping method, *A. caviae* was found in irrigation water (Latif-Eugenin *et al.* 2016). *A. caviae* was the most prevalent in meat product, based on its HKG-like *rpoD* and *gyrB* sequences (Nagar *et al.* 2013). Eel is also a reservoir of *A. caviae* among many other *Aeromonas* genus (Yi *et al.* 2013). A study in Taiwan showed *A. caviae* was recovered from suspected food-poisoning samples from retail market. These samples including seafood, rice food, vegetables, and fruits. Swabs from kitchen and cooker hands were also a reservoir for *A. caviae* (Chang *et al.* 2008). These reservoirs of *A. caviae* source are related to the food consumed by human. In other hand, kitchen sponge is needed to clean the food utensils at restaurant, retail shop, and household. Moreover, *A. caviae* was the most frequent species, among other *Aeromonas*, isolated from the human clinical specimens (Fernandez-Bravo and Figueras 2020). Those sources including ten different human specimens. *A. caviae* has been associated with blood infections, causing septicemia. Antimicrobial resistance recently becomes a worldwide threat, due to difficult to combat. Moreover, *A. caviae* has been reported to express class C and D β-lactamases, which considers as a resistance action to several antibiotics (Janda and Abbott 2010). The presence of virulence genes in pathogenic bacteria lead to elevating the disease development. Virulence genes presentation of *A. caviae* from food samples in Taiwan became a concern since this strain harbored virulence genes, sucah as heat-labile cytotonic enterotoxin (Alt), heat-stable cytotonic enterotoxin (Ast), and cytotonic enterotoxin (Act). These virulence genes were associated to diarrhea caused by *Aeromonas* spp. (Chang *et al.* 2008).

BLASTn result of KS-1 sequence showed several genera *Aeromonas* which had a high identity based on 16S rRNA. *A. caviae* IR11 was isolated from gill and intestine of muller fish (*Mugis cephalus*) from Indian West Coast. Out of these isolates, two from thirty four isolates had α hemolytic activity. *A. dhakensis* W2-2 was isolated from sediment of aquaculture fish pond in China. *Aeromonas* spp. became the predominant genus (29/74). This study showed *qnrS* gene, responsible for quinolone resistant, was detected in 50% isolates using PCR. *A. hydrophila* T50-2 was isolated from aerobic biofilm reactor under oxytetracycline dosage. In this study, they found a novel transposon Tn6433 with tet(E).

Moreover, this tet(E) was found in both chromosome and plasmid. *A. punctata* WAB 1957 was isolated from Tiete River in Sao Paulo, Brazil. This isolate was grown in Salmonella-Shigella agar. Antimicrobial susceptibility study showed this isolate is sensitive to erihtomycin, ampicillin, and vancomycin, while it is resistant to gentamycin.

Omphisa fuscidentalis can be an alternative for *G. mellonella* as a bacterial infection model (Ilsan *et al.* 2023). Moreover, *O. fuscidentalis*-injected larvae with *Aeromonas* spp. showed several changes starting from 5 hours after injection either in melanization score or survival pecentage. It is different with *G. mellonella* as the changes started from 18-24 hours after *A. caviae* injection (Ilsan *et al.* 2021). The graded concentration dosage of bacterial injection showed a linear identical trend for survival and melanization score.

Insect model comprise two major immune systems, including cellular and humoral immune system. The cellular immune system in the larvae is mediated by phagocyte cells, called hemocyte, in hemolymph. The hemolymph is an analogue to the mammalian blood. Hemocyte in larvae is not only has a phagocytic activity, but also encapsulating and clotting the foreign invaders. The humoral immune system is mediated by soluble chemicals to trap the bacteria, besides acting as complement-like substances, antimicrobial peptides, and melanin (Tsai *et al.* 2016). Melanization or blackness in *O. fuscidentalis* larvae-injected by *Aeromonas* spp. was likely caused by deposition and synthesis of melanin to trap or encapsulate the bacteria together with hemolymph opsonization and coagulation. This phenomenon was closely related to abscess development in bacterial infections in mammalian. Melanin is developed by phenoloxidase in hemocyte. Phenoloxidase also has been known to produce reactive oxygen species that can damage the bacteria.

Suprisingly, in 24 hours after injection of *O. fuscidentalis* model, the survival of PBS-injected as a negative control, was 80%. The dead larvae did not show blackness or melanization. It is likely because the temperature 37°C used for incubation. It is different with those *G. mellonella* larvae that could live in 37°C for several days (Ilsan *et al.* 2021, 2023). Health status of larvae after bacterial injection in *O. fuscidentalis*, was in a higher resolution observed by melanization score than larvae's survival.

We observed the changes in morphology of hemocyte in *Aeromonas* spp. injected-larvae. Bigger vacuole and more vacuolization of hemocyte were evaluated, compared to the PBS-injected hemocyte. The study of *G. mellonella* larvae injected by *Pseudomonas aeruginosa* culture showed significant changes in viability and morphology of hemocytes, including plasmatocytes and granulocytes. The hemocyte changes including membrane blebbing, cell and organelle swelling, condensation of chromatin, and vacuolization in cytoplasma. This morphology tends to become autophagy and apoptotic activity to the cell death. The staining further by propidium iodide and acridine orange showed that the hemocyte become dead. The cohesion of melanization and hemocyte changes likely cause larvae dead.

In conclusion, *Aeromonas* spp. from 2-week used household kitchen sponge was isolated, then identified based on its 16S rRNA sequence. As an alternative of *Galleria mellonella* larvae as an infection model, *Omphisa fuscidentalis* that has similar taxonomic order was used. Graded concentration of bacterial dosage of 10^4-10^7 CFU/larva was injected to *O. fuscidentalis* larvae. Larvae with the highest infection dosage was inversly proportional to the *O. fuscidentalis* survival percentage. Interestingly, the melanization score evaluation gained more resolution than a survival percentage, although both resulting the linear healthy status against the graded bacterial dosage. Furthermore, in hemocyte observation under microscope, larvae with melanization score 1 had larger and more cytoplasmic vacuolization compared to larvae with melanization score 4 or healthy larvae. This study brings a new insight of *O. fuscidentalis* as an alternative animal model for bacterial infections.

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